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HYBRIDIZATION OF KARYOTYPICALLY DIFFERENTIATED POPULATIONS IN THE *SCELOPORUS GRAMMICUS* COMPLEX (IGUANIDAE)

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Among the approximately 60 species of the lizard genus *Sceloporus*, there is much karyotypic diversity, resulting primarily from the fixation of "Robertsonian rearrangements" (centric fusions and fissions; Hsu and Mead, [1969](#); Jackson, [1971](#)). Diploid ($2n$) chromosome numbers vary from 22 to 46 (Cole, [1970](#), [1971](#); Hall, [1973](#)), a range exceeding that known for any other lizard genus. Because comparable variation has been detected in several groups of vertebrates, including other lizard genera (Gorman and Atkins, [1968](#); Matthey and van Brink, [1960](#)) and rodents (review by Nadler, [1969](#)), and in many invertebrates, evolutionists are becoming increasingly concerned with the role of karyotypic changes in animal speciation (White, [1969](#); Mayr, [1969](#), [1970](#)).

To investigate relationships between karyotypic diversification and speciation, Hall has undertaken a survey of chromosomal variation in *Sceloporus* and related genera, seeking particularly to identify intermediate evolutionary stages. Of 48 species of *Sceloporus* examined, all but one, the Mexican form *S. grammicus*, are karyotypically monomorphic, at least for Robertsonian mutations of the autosomes. An analysis of 1200 individuals of *S. grammicus* has revealed extensive chromosomal diversity at several levels of population structure, including the occurrence of rare individual variants,

polymorphism, and the fixation of different Robertsonian modifications in parapatrically or allopatrically distributed populations. Chromosome numbers vary geographically from $2n = 31$ to $2n = 46$. The available data on karyotypic variation and geographic and ecologic distributions of populations suggest that the morphologically defined taxon *S. grammicus* (Smith, 1939; Smith and Laufe, 1945) actually represents several semispecies or cryptic species in early stages of differentiation. In the present study we have examined patterns of karyotypic and genic variation in a zone of contact between two karyotypically distinctive populations of *grammicus* to determine the extent of their genetic isolation. This research has implications for the systematic problem of defining species limits in the *grammicus* complex and contributes to an understanding of the evolutionary significance of parapatric hybridization.

KARYOTYPIC VARIATION IN THE *GRAMMICUS* COMPLEX

A comprehensive analysis of the karyology and of the geographic, ecologic, and evolutionary relationships of the *grammicus* complex will be presented elsewhere (Hall, [1973](#); Hall and Alvarez-S., [1973](#)), but a summary here will be useful.

A common karyotype in *grammicus*, designated Standard (S), includes six pairs of metacentric macrochromosomes and, in the female, 10 pairs of microchromosomes (Fig. [1A](#)). Males with the S karyotype have only 19 microchromosomes due to the presence of an X_1X_2Y sex chromosome trivalent (White, [1954](#)), which also occurs in the *grammicus* populations with differing karyotypes. Since very similar or identical karyotypes are found in the eight karyotyped species of the *torquatus* species group of *Sceloporus* (Cole et al., [1967](#); Hall, [1973](#)), two species of the *megalepidurus* group, and the remaining two species of the *grammicus* group (Hall, [1973](#)), we suggest that the S pattern is primitive in the evolution of *grammicus* karyotypes. This suggestion is supported by the wide and apparently disjunct distribution of the S karyotype over much of the range inhabited by *grammicus* (Fig. [2](#)). All other karyotypes in the *grammicus* complex would then appear to have been derived from the S pattern by various sequences of centric fissioning, a process firmly documented in the Iguanidae (Webster et al., [1972b](#)).

Polymorphic-1 (P1) populations differ from those of S only by being

polymorphic for a centric fission of chromosome 1 (Fis-1) (Fig. 1B). The Fis-1 mutation is limited to populations occurring above 3200 m elevation on the chain of three mountains (Tlaloc, Ixtaccihuatl, and Popocateptl) forming the eastern boundary of the Valley of Mexico (Fig. 3A). These populations occupy a range of perhaps less than 500 km² and probably consist of between S X 104 and S X 105 individuals. In a sample of 289 karyotyped lizards from this area, the frequency of the Fis-1 mutation was 0.108.

The *Fission-6* (F6) karyotype, characterized by fixation of a centric fission of chromosome 6 (Fis-6) and a female 2n = 34 (Fig. 1C), occurs in populations in the Sierra Madre Oriental of Nuevo Leon, Tamaulipas, and San Luis Potosi, and in the Sierra Volcanica Transversal between western Michoacan and the Valley of Mexico (Fig. 2). On the eastern side of the Valley of Mexico, an F6 population occurs between S populations found below 2400 m and P1 populations occurring above 3200 m. Farther west, a disjunct population of F6 occurs on the Nevado de Colima, where its range is probably less than 70 km².

Three other karyotypic systems have been found in grammicus (Fig. 2). Populations of F5+6 are monomorphic for fissions of chromosomes 5 and 6; those of FM (multiple fissions) are monomorphic for fissions of chromosomes 2, 4, 5, 6, and a micro-chromosome, and are polymorphic for fissions of chromosomes 1 and 3; and populations of F5 are monomorphic for a fission of chromosome 5.

ZONE OF CONTACT BETWEEN P1 AND F6

Although there appear to be no ecologic or geographic barriers between many of the karyotypically distinctive grammicus populations, our survey has provided no evidence of sympatry or of intergradation through clinal changes in frequencies of chromosomal types. However, several parapatric contacts, all involving very narrow zones of hybridization, have been found in or near the Valley of Mexico: between S and F6 populations near Cuernavaca (Fig. 3A); between S and FM in the Archeological Zone around the Pyramids of Teotihuacan (Fig. 3A); and between P1 and F6 near Amecameca (Fig. 3A) and several places near Rio Frio (Fig. 3B).

Karyotypic variation through the zone of contact between P1 and F6 populations has been studied along five transects on the western slope of the

eastern divide of the Valley of Mexico and one on the eastern slope (Fig. 3). Collection sites along three of the western transects (Amecameca, Arroyo Dos Aguitas, and Cerro Potrero) were spaced closely enough to pinpoint the contact zone, as indicated by the occurrence of individuals heterozygous for the Fis-6 mutation. Three were found in the transect east of Amecameca (made in the summer of 1969) (Fig. 3A); 13 were collected in the transect along the Arroyo DOS Aguitas (1970) (Fig. 3B); and two were obtained near Cerro Papayo (1970) (Fig. 3B). These heterozygous individuals demonstrated that P1 and F6 individuals can successfully interbreed to produce at least an F_1 generation, but the narrowness of the zone in which heterozygotes were found suggested that introgression is severely limited. Another transect made in the summers of 1968 and 1969 around the northwestern corner of the Llano Grande (a large meadow) (Fig. 3B) yielded no heterozygotes, presumably because individuals collected in the critical area within the Canada del Quesero were not karyotyped; but "pure" P1 and F6 samples were taken within 200 m of one another. In none of the transects was the zone of heterozygosity more than 400 m wide, a distance perhaps equal to the dispersal distance of individuals. We found no individuals homozygous for the Fis-6 mutation which also carried the Fis-1 mutation.

Below the zones of contact, which lie between 3000 and 3400 m elevation, F6 populations usually inhabit dense, relatively humid forest in which oak, pine, and fir are important components. In contrast, P1 populations occupy the relatively uniform open pine woodland that extends upward from the humid forest to tree-line. Ground cover in the humid forest consists of dense herbs, while that of the pine woodland is short grass interspersed with clumps of bunchgrass. Hence, the environments of the P1 and F6 populations differ, even though both forms have a preferred structural habitat of logs and stumps. But the habitat difference is not absolute, for F6 populations occur locally in pine woodland similar to that normally occupied by P1 populations, and, conversely, P1 populations may be found in stands of humid forest. For example, the area inhabited by F6 west of Llano Grande is reasonably typical pine woodland, and the pine forest east of the Llano, where P1 individuals are common, is unusually thick and locally contains many oaks (Fig. 3B).

To examine more thoroughly the nature of the zone of hybridization between P1 and F6 populations, a new transect was established on the southwestern slope of Cerro Potrero in the latter part of summer, 1970 (Fig. 3B). We located the zone in this area by karyotyping single individuals collected at

500-m intervals along a trail crossing the area of transition from humid forest to pine woodland. This exploratory survey yielded one Fis-6 heterozygote from the area of transition, together with P1 individuals from the pine woodland and F6 individuals from the humid forest. For karyotypic and allozymic studies, we then collected 164 lizards, representing about 50% of the population, in this 600 X 500 m, approximately cruciform area centered on the collection site of the Fis-6 heterozygote.

METHODS AND MATERIALS

Karyotypes

Karyotypes were determined by methods similar to those of Evans et al. (1964) and Patton (1967). Dividing cells were obtained from testes and/or bone marrow and spleen. Only in the case of the sample from the Cerro Potrero transect were the same individuals both karyotyped and electrophoresed.

Allozymic Analysis

Methods of tissue preparation, starch-gel electrophoresis, and protein staining were similar to those described by Selander et al. (1971), as modified for lizards by Webster et al. (1972b) and McKinney et al. (1972). Most of the proteins were demonstrated in aqueous extracts of the whole body, but hemoglobin and transferrin were stained in hemolysate and plasma, respectively. Alleles at each polymorphic locus were designated alphabetically in order of decreasing rate of electrophoretic mobility of their corresponding allozymes. Migration was anodal for most proteins but cathodal for ADH-1, PGI-1, and GOT-2.

Formal genetic analysis by progeny testing has not been made for *grammicus*, but our interpretations of polymorphic variation are supported by several lines of indirect evidence. First, the banding patterns of most proteins in *Sceloporus* are similar to those of homologous proteins in other vertebrates for which extensive genetic data are available from progeny studies (Lush, 1970). Second, in samples of local populations (apart from those in the zone of hybridization), we find no significant deviation from proportions of phenotypes (and presumed genotypes) expected on the basis of the Hardy-Weinberg theorem (with correction for bias in small samples; Levene, 1949).

Third, the correlation of allozymic and karyotypic intermediacy in individuals of the hybridizing population at Cerro Potrero supports the interpretation that the polymorphisms are controlled by segregation of alleles at single loci. Similarly, a genetic basis for allozymic variation in hemoglobin, albumin, and transferrin has been documented in the iguanid lizard genus *Anolis* by an analysis of hybrids (Gorman et al., [1971](#)).

Samples

Genic variability samples.--To determine degrees of overall genic similarity and to identify loci useful in analyzing hybridization between populations of P1 and F6, variation in proteins encoded by 19 or 20 loci was surveyed electrophoretically in samples of one P1 population and three F6 populations, as follows:

- *P1: NE of Llano Grande, Mexico; 4 km W, 1.7 km S Rio Frio, 3200-3250 m, August, 1970; N = 31. A sample of 54 individuals taken at this locality in September and October, 1968, was karyotypically P1, as was an additional sample of six karyotyped specimens from the 1970 sample.*
- *F6: SW of Llano Grande, Mexico; 5.9-6.5 km W, 3.0-4.5 km S Rio Frio, 3160-3220 m, August, 1970, N = 37. All 18 individuals collected at this locality and between it and the Llano Grande in 1968 and 1969 were karyotypically F6, as were two additional karyotyped specimens from the 1970 sample. Cerro de Garnica, Michoacan; 21 km WSW Ciudad Hidalgo, 2890 m, July, 1970; N = 21. Karyotypes were determined for four individuals. Nevada de Colima, Jalisco; July, 1970; N = 30. Five small samples were collected at localities 1 to 2 km apart between 3000 and 3300 m on the northern slope of the mountain. Eight individuals from these samples were karyotyped.*
- *Cerro Potrero Transect: SW slope Cerro Potrero, Mexico; 7.2-7.6 km W, 1.4-2.2 km S Rio Frio, 3350-3450 m, August-September, 1970; N = 153. All individuals were both karyotyped and electrophoresed, and karyotypes were determined for an additional 11 individuals. Only those structural gene loci at which the P1 and F6 samples from the Llano Grande area differed markedly in allele frequencies were assayed electrophoretically.*

To test for possible geographic variation, these polymorphic structural loci were studied electrophoretically in an additional P1 sample:

- *Cerro Las Tejas*, Mexico; a series of collections from an area 8.5-11.0 km SSW Rio Frio, 3400-3600 m, September, 1970; N = 28.

RESULTS

Allozymic Variation

Allele frequencies at 19 structural gene loci in four samples of F6 and at 20 loci in the sample of P1 from NE Llano Grande are presented in [Table 1](#). For a few of the proteins studied, special features of variation require description and comment, as follows:

Esterase-1.--This protein, which was demonstrated both in extracts of the whole body and in plasma, is the only prominent esterase that is inhibited by eserine. Variation involves the presence or absence of a band and is interpreted as the product of two alleles, one of which (*Es-1^a*) is "silent." Allele frequencies were estimated from the proportion of individuals apparently homozygous for the *Es-1^a* allele, with appropriate correction for bias in small samples (Haldane, [1956](#)).

α-Glycerophosphate dehydrogenase.--In all populations of the *grammicus* complex except P1, allozymic variation in αGPD is unexceptional; homozygotes have a single band and heterozygotes a three-banded phenotype, with the "hybrid" or hetero-dimer band especially prominent. But all 28 P1 individuals from Cerro Las Tejas and 29 of the 31 P1 specimens from NE Llano Grande have a three-banded phenotype; and the two remaining individuals from NE Llano Grande have five- or six-banded phenotypes. Apparently the *αGpd-1* locus has been duplicated in the P1 population, establishing a universal "heterozygous" condition, with alternate alleles being nearly fixed at the two loci. The five- or six-banded patterns presumably reflect heterozygosity at one locus and homozygosity at the other. We have designated the locus with the high frequency of the *c* allele as *αGpd-1A* and that with the high frequency of the *b* allele as *αGpd-1B* ([Table 1](#)); and we have provisionally assigned the *a* allele to the *αGpd-1B* locus and the *d* allele to the *αGpd-1A* locus. Although this gene duplication potentially provides a marker for hybrid genotypes, variation in αGPD was not used in analyzing hybridization in the contact zone because we could not reliably distinguish the permanent heterozygosity of the P1 condition from the various types of heterozygotes produced by hybridization.

Albumin.-Polymorphism in albumin was observed in most of the *grammicus* populations, but, because we could not reliably determine homologies of alleles from different localities, we scored this protein only in samples from the Rio Frio study area. Four alleles were identified in this material ([Table 1](#)).

Genic Heterozygosity and Similarity

Estimates of proportions of loci polymorphic per population and heterozygous per individual are presented for populations of P1 and F6 in [Table 2](#). These estimates are based on 19 loci (neither the *α Gpd-1B* nor the *Alb-1* locus was included). Average heterozygosity (H) for the four populations is 0.070, a value similar to those reported for *Uta stansburiana* (0.05) (McKinney et al., [1972](#)) and *Anolis carolinensis* (0.06) (Webster et al., [1972b](#)). If additional data from our small samples of S, F5 + 6, and FM populations are included, $H = 0.072$. Variation among populations is marked, with conspicuously low heterozygosity in the P1 population and in the F6 population from the Nevado de Colima, both of which occupy relatively small areas. Overall genic similarity between paired combinations of samples was measured by Rogers' coefficient (S) (Rogers, [1972](#)), based on allele frequencies at 20 loci ([Table 3](#)). For the Llano Grande P1 and F6 populations, $S = 0.73$. Surprisingly, the degree of genic similarity with P1 increases in F6 populations to the west of the Valley of Mexico, being 0.79 for Cerro Garnica and 0.84 for Nevado de Colima. Among pairs of F6 populations, S averages 0.89.

Previous studies indicate that S values are generally larger than 0.85 for conspecific continental populations of lizards and other vertebrates (Avice and Selander, [1972](#)). For example, among populations of the lizard *Uta stansburiana* in the western United States, $S = 0.89$ (data presented by McKinney et al., [1972](#)). Lesser degrees of similarity are reported for strongly isolated but presumably conspecific populations, for example, between insular and continental populations of *Anolis carolinensis* ($S = 0.69$) (Webster et al., [1972b](#)). Comparing four nonsibling species of *Anolis* occurring on Bimini Island, Webster et al. ([1972b](#)) found that genic similarity averaged only 0.21 (range, 0.16 to 0.29).

Genetic Analysis of the Cerro Potrero Hybrid Zone

Genic and karyotypic data for all electrophoretically analyzed samples from

the Rio Frio study area (Fig. 3B) are summarized in Table 4. "Fixed differences" are chromosomal and genic variants that are differentially monomorphic in P1 and F6 populations near the contact zone.² "Polymorphic differences" refer to chromosomal and genic characters, shared variants of which occur in the parental populations.

Theoretically, F₁ hybrids carry unrecombined haploid genomes derived from the P1 and F6 parental populations, and should therefore be heterozygous for all three fixed differences. Of the 153 individuals from the Cerro Potrero transect, 13 (seven males, six females) are presumptive F₁ hybrids by this criterion. Additionally, 56 individuals (32 males, 24 females) are heterozygous for only one or two of the markers, a fact indicating that hybridization is not limited to the formation of an F₁ generation.

Provided that alleles³ at the three marker loci assort independently in the gametogenesis of F₁ hybrids, each marker locus in a first generation backcross (B₁) individual has a 0.5 probability of being heterozygous. For three markers, B₁ individuals may be heterozygous at all three loci, two loci, one locus, or none; and these classes should occur in the ratio of 1:3:3:1. Triple and zero heterozygotes would, of course, be indistinguishable from F₁ hybrids and parental types, respectively. If the single and double heterozygotes collected in the Cerro Potrero transect represent only a B₁ generation, they should occur with approximately equal frequency in collections. But further backcrossing (introgression) should produce an excess of individuals heterozygous for one marker.

In the "backcross to F6" category (Table 4), the numbers of single and double heterozygotes are similar (13 and 14, respectively). But in the "backcross to P1" category, single heterozygotes are perhaps more frequent than double heterozygotes (adjusted $\chi^2_{(1)} = 3.448$, $p \approx 0.06$), a circumstance that might be interpreted as evidence of introgression into the P1 population. However, it should be noted that the frequencies of the three genotypic arrays of the double heterozygous class are also unequal ($\chi^2_{(2)} = 8.00$; $p \approx 0.025$). If the observed deficiencies in the genotypic arrays heterozygous for *Got-1* and *Ldh-2* and for *Got-1* and *Fis-6* are real, they could account for the relatively low frequency of double heterozygotes in the "backcross to P1" category. In view

of this possibility, the evidence for introgression provided by the fixed differences is equivocal, at best. Although the numbers of individuals in the double and single heterozygote classes in the "backcross to F6" category are approximately equal, one genotypic array in each of these classes also seems deficient, but the observed deviations are not statistically significant at conventional levels of probability (for double heterozygotes, $\chi^2_{(2)} = 5.281$, $p \approx 0.07$; and for single heterozygotes, $\chi^2_{(2)} = 4.311$, $p \approx 0.1$). If the apparent deficiencies of individuals with these genotypic arrays involved related genotypic classes in the two backcross categories, they might be attributed to linkage of certain of the marker loci, but they do not. Nor do the deficiencies seem to correlate with heterozygosity for specific markers. Rather, the observations in the backcrosses to both parental types suggest that the deviations in frequency result from backcross breakdown; that is, certain recombination products of the F₁ hybrid genome paired with the haploid genomes of the parental types may have relatively low viability.

If the presence of only one recombined hybrid genome in a backcross genotype is deleterious, the presence of two such re-combined genomes in an F₂ hybrid zygote must be considerably more deleterious; so we would expect few, if any, of these F₂ progeny to survive. On the other hand, the discovery of identifiable F₂ individuals would tend to refute the thesis of backcross breakdown. In the present case, assuming independent assortment of alleles at three heterozygous marker loci in the F₁ parents, 3/8 of the F₂ progeny should be homozygous F6 parental type at one locus and homozygous P1 parental type at another locus. These genotypes would be distinctive, as they could not result from backcrossing; but none was found. Since up to 1/3 of the individuals taken from given log piles near the center of the hybrid zone in the transect area were F₁ hybrids (Moody et al., [1973](#)), F₂ matings would seem quite possible. The discovery of any individuals with distinctive F₂ genotypes would have considerably weakened our supposition that genetic breakdown is occurring in the back-cross generations. However, our sample from the transect is too small for the absence of these genotypes to provide significant support of the thesis.

The observed deviations of frequencies for the arrays of fixed differences ([Table 4](#)) from those predicted by simple back-crossing and introgression models might result from sampling only a few introgressive families.

However, detailed analyses of population structure and the micro-geographic distribution of genotypes within the transect area by Moody et al. (1973) provide no evidence for this. About half of the individuals used in the present study were collected at sites sufficiently separated from one another that biased sampling of individual families was unlikely. Two of the four single individuals representing markedly deficient genotypic arrays came from scattered collections of this type. The other two individuals representing deficient genotypic arrays came from large samples at single localities, respectively containing six and eight other backcrosses in the same directions besides other hybrid and parental types. If these two backcross samples each represent only the progeny of single back-cross matings, the deficiencies still suggest backcross breakdown.

Analysis of the allele frequencies at the various polymorphic loci provides another approach for understanding the genetics of hybridization in the transect area.

Because F_1 hybrids carry one haploid genome from each parent, the frequency of alleles at polymorphic loci in the F_1 class should be intermediate between frequencies in the parental populations. Similarly, in the B_1 generation, frequencies may be expected to fall midway between the F_1 hybrid and parental frequencies. Provided that the alleles at the polymorphic loci assort independently from those of the fixed differences and that the backcrosses represent only a B_1 generation, the frequencies of alleles at polymorphic loci should be about equal in the single and the double heterozygote categories established by the three marker loci. But if some of the polymorphic loci are linked with those showing fixed differences, or if generations of backcrossing beyond the B_1 occur, allele frequencies at the polymorphic loci in the single heterozygote categories should more often resemble those of the parental type than should those in the double heterozygote classes.

In the "backcross to P1" category, allele frequencies at all five polymorphic loci are more like those of "pure" P1 samples in the single heterozygotes than in the double heterozygotes. And in the "backcross to F6" category, for three of five loci the single heterozygotes are in this regard more like the "pure" F6 than are the double. The probability that frequencies at all five loci in the "backcross to P1" category would deviate in the same direction by chance is 0.03 (one-tailed Sign Test). In the "backcross to F6" category, the probability

that three of five would show the same deviation is 0.12. In testing for linkage, deviations in the backcrosses to the two parental types are not considered independent events, and, in this case, eight of 10 deviations are in the expected direction ($p = 0.004$).

The consistent differences in allele frequencies at the five polymorphic loci between the single and double heterozygote classes in the "backcross to P1" category suggest that some of the individuals in the single heterozygote class may represent B_2 or further generations of backcrossing. This interpretation is supported by the aforementioned disproportion in numbers of individuals in the single and double heterozygote classes, to the extent that this disproportion is not due to backcross breakdown. However, the fact that allele frequencies at the polymorphic loci in the class of "pure" P1 individuals (those homozygous for the three marker loci) from the Cerro Potrero transect are closely similar to those of samples taken one to three kilometers back from the zone of contact (NE Llano Grande and Cerro Las Tejas) ([Table 4](#)) clearly demonstrates that the present rate of introgression at these polymorphic loci is at most very low. (Past introgression may account for the presence at low frequencies in the P1 gene pool of the four genic alleles occurring in the F6 population.) For the three fixed differences there is no evidence of introgression into P1 populations beyond the Cerro Potrero transect area. In the F6 populations, one individual heterozygous for two of these markers was found in the SW Llano Grande sample, but this is most reasonably treated as an immigrant F_1 or B_1 rather than as evidence for introgression (see [Note 2](#)).

An alternative hypothesis explaining the observed variation at the polymorphic loci can be developed on the assumption that several of the loci are closely linked with those of the fixed differences, but, unfortunately, linkage groups cannot be established from the available data.

In sum, our analysis of genetic variation in the sample from the Cerro Potrero transect demonstrates that P1 and F6 populations interbreed to produce viable F_1 hybrids, which, in turn, successfully mate with both parental types to produce B_1 back-cross generations. The extreme narrowness of the hybrid zone, the lack of evidence for the formation of F_2 or B_2 and further backcrosses with F6, and the at best equivocal evidence for only a small amount of introgression into P1 suggest that most surviving B_1 individuals

are effectively sterile. Less certainly, the observation that some B_1 genotypic arrays occur less frequently than expected suggests that certain gene combinations in linkage groups including at least some of the marker loci have low viability. Other possible but less likely explanations for these deviations (e.g., limited introgression, biased sampling of too few families, etc.) cannot be ruled out because of our small sample size. However, our data and analyses clearly show that the genetic structure of the hybrid zone between the P1 and F6 populations may be determined to a high degree of precision by more extensively sampling undisturbed areas of the zone. Controlled hybridization experiments and analytical studies of meiosis in hybrids and back-crosses should reveal the reasons for this structure.

DISCUSSION

We have shown that P1 and F6 populations of *grammicus* meet along a broad front and interbreed in a zone approximately 400 m wide passing through the Cerro Potrero area, and the hybridization is largely, and perhaps entirely, limited to the formation of F_i and B_i generations. The concept of hybrid inferiority, and particularly that involving reduced viability or fertility in backcross generations, has often been invoked to explain the extreme narrowness of zones of hybridization between parapatric populations (Mayr, [1963](#); Hagen, [1967](#); Yang and Selander, [1968](#)), but rarely have supporting genetic data been marshaled for animals (Dobzhansky, [1970](#)). Our evidence is circumstantial, however, and does not eliminate the need for more direct demonstrations of selection against hybrid recombination products (Hagen, [1967](#)).

Despite hybridization, genes of P1 apparently are not introgressing into populations of F6; and, if in fact there is some introgression of F6 genes, the effect on the gene pool of the P1 population is very slight. Absolute sterility of B_1 individuals need not be invoked as a barrier to gene flow, since there are many examples of an absence of introgression through comparable zones of hybridization, even when there is some fertility in the B_1 generation (Mayr, 1963; Hagen, [1967](#); Hunt and Selander, [1973](#)). In any event, the considerable difference between the P1 and F6 populations reflected in estimates of genic distance and of degree of genic heterozygosity suggests that they are evolving independently. Hence, distinctive genetic systems are presently being maintained in close geographic proximity, notwithstanding an absence of effective prezygotic isolating mechanisms.

From a systematic standpoint, the important criterion of species status is not the absence of interbreeding per se but, rather, of genetic exchange between populations. The concept that genetic isolation cannot be directly equated with reproductive isolation, as developed by Stebbins (1950), Bigelow (1965), and Hagen and McPhail (1970), emphasizes the fact that strongly integrated and coadapted gene pools may be effectively protected against introgression (and thus evolve independently) even in the absence of reproductive isolation (Mayr, 1970). By the criterion of genetic isolation, the P1 and F6 populations of the *Sceloporus grammicus* complex are biological species.

Remington (1968) has proposed that all zones of parapatric hybridization are recent (a few hundred to a few thousand years old) and that the frequency of hybridization occurring in zones of secondary contact "rather rapidly" declines due to the establishment of anti-hybridization mechanisms. But this thesis is supported neither by relevant theory (Moore, 1957) nor by empirical evidence (Short, 1969; Mayr, 1970; Selander, 1971). Extensive evidence from avian studies suggests that most zones of hybridization are ancient, dating from Wisconsin and even pre-Wisconsin times (Short, 1970). Because there is no reason to believe that hybridization between P1 and F6 grammicus populations was only recently established, we have sought to interpret the situation in terms of an equilibrium between potentially introgressive gene flow and counter selection. The humid forest and the pine woodland inhabited by F6 and P1 populations, respectively, are native vegetation types that have existed in the mountains east of the Valley of Mexico for thousands of years (although they have likely varied in relative extent), as demonstrated by pollen core analyses from the floor of the eastern part of the Valley (Clisby and Sears, 1955; Sears and Clisby, 1955; Gonzalez-Q., 1970). And there is no evidence that the structure and distribution of these vegetation types have been altered by human activity to such a degree that the position or extent of the contact zone between the two populations has been affected. Populations of F6 probably have been continuously present in the Rio Frio area for many thousands of years; this region is the center of a widespread and presumably ancient distribution extending from the Nevado de Colima to northern Nuevo Leon (Fig. 2).

The origin and evolutionary history of the P1 population are uncertain. If P1 was derived directly from an S ancestor, as the nature of its karyotype might suggest, then we may presume that it either represents the original inhabitant

of the eastern divide of the Valley of Mexico or that it reached its present geographic position by penetrating the F6 population, possibly at a time when the distribution of F6 was relatively restricted in these mountains. (In either case, it is likely that the P1 and F6 populations have been in parapatric contact throughout much of their evolutionary histories.) Unfortunately, we do not presently have comparative genic data for populations of S in the region of the Valley of Mexico ([Fig. 2](#)) that may have shared a common ancestry with the P1 population. The demonstration of close genic similarity between these forms would strengthen the hypothesis of a derivation of P1 from S suggested by their karyotypic similarities.

An alternate hypothesis deriving P1 from within the F6 radiation might be advanced on the basis of the marked genic similarity of P1 and the geographically isolated population of F6 on Nevado de Colima ($S = .84$). But this evolutionary scheme involves two karyotypic modifications, fusion of the two acrocentric 6 elements and fissioning of chromosome 1, whereas only the second event is involved in deriving P1 from S.

Whether derived from F6 or from S, it is likely that evolution of the P1 population occurred in geographic isolation and that its present contact with F6 is secondary. A population similar to F6 may have been isolated from the main body of the species in a period of relative aridity, when the oak, fir, or other mesic vegetation types now occupied by F6 were severely restricted in extent or absent in the region of the Valley of Mexico. This is most likely to have occurred during the Thermal Optimum, from ~ 10,000 to 7,000 BP, when the lakes in the Valley of Mexico apparently evaporated and oak and fir pollen were not being deposited on the floor of the Valley (Clisby and Sears, [1955](#); Maldonado-K., [1964](#)). Confined to higher elevations, the proto-P1 population presumably adapted to the open pine woodland which it presently inhabits. Following the Thermal Optimum, the F6 population may have either expanded its range from small refugia or mesic forest in the mountains east of the Valley of Mexico or reinvaded the area from the west. In any event, F6 came to surround the P1 population, establishing contact and hybridizing in a narrow zone. According to this reconstruction of events, P1 is a relic population that in geographic isolation differentiated sufficiently to prevent "swamping" by introgressive genes of F6 once secondary contact was effected.

Provided that the zone of contact between P1 and F6 is in fact approximately 7,000 years old, the failure of premating isolation to evolve is perhaps somewhat paradoxical, especially since the present P1 population is so small

and its range is so narrow that perhaps 10% of all individuals of P1 occur within dispersal distance of the zone. Presumably P1 would not experience the difficulties of coadaptive selection that are likely to prevent the reinforcement of isolating mechanisms in more widely distributed species meeting in zones of secondary contact (Moore, [1957](#)).

We note that Hall's studies indicate a general failure of premating isolation between other forms of the *grammicus* complex hybridizing in similarly narrow zones.

The analysis of the allopatrically distributed sibling species of the *grammicus* complex in Mexico has thus far provided little insight into the role of karyotypic changes in speciation. Although it seems likely that species limits in the complex are coordinate with the karyotypic variants, it yet remains to be determined if chromosomal modifications themselves have functioned in any causal way in relation to species formation, either by the spread of chromosomal mutants through continuously distributed populations (White, [1969](#)) or according to other models of "chromosomal speciation," including those involving the fixation of chromosomal variants in small, isolated populations (Mayr, [1969](#)). In the case of P1 and F6, geographic isolation of moderately large populations (especially likely as far as F6 is concerned), followed by secondary contact after chromosomal and genic differentiation had occurred, seems plausible. Whether the apparent hybrid breakdown is entirely genic or in part chromosomal (perhaps involving cryptic structural differences as well as the obvious Robertsonian modifications) remains to be determined (see discussion in Dobzhansky, [1970](#)).

SUMMARY

The Mexican lizard *Sceloporus grammicus* consists of six parapatric populations differing by one or more fixed and/or polymorphic centric fissions from the primitive *grammicus* karyotype (Standard) of 12 metacentric macrochromosomes and 20 microchromosomes (in the female). Very narrow zones of parapatric hybridization occur within the Valley of Mexico between three pairs of these karyotypically differentiated populations.

Allozymic variation at 19 or 20 gene loci was examined in the Fission-6 (F6) and Polymorphic-1 (P1) populations, which hybridize in a zone 400 m wide on the east side of the Valley of Mexico. Two F6 populations, one at the

contact zone and one about 200 km west of the zone (both in the continuous part of the range of F6) were genically rather variable-- $H = 0.105$ and 0.134 , respectively; while the P1 and a disjunct F6 population, both of which are small, showed low variability-- $H = 0.024$ and 0.017 , respectively. P1 and F6 samples from the contact zone showed low genic similarity ($S = 0.73$) relative to the similarity among pairs of F6 populations ($S = 0.89$). Surprisingly, S for P1 with F6 increases to the west, being 0.84 for the comparison with the westernmost F6 population (Nevado de Colima).

Electrophoretic and karyological analysis of 153 individuals from a transect through the P1 X F6 hybrid zone at Cerro Potrero revealed 13 presumptive F_1 hybrids, 29 probable backcrosses to P1, and 27 probable backcrosses to F6. There is no apparent introgression from P1 into F6, but a low level of introgression from F6 into P1 may be occurring. Deficiencies of certain genotypic arrays suggest that some recombination products in the backcrosses survive poorly, and that those B_1 individuals that survive to maturity do not reproduce. The genic similarity and heterozygosity values are in accord with the interpretation that F6 and P1 are independently evolving biological species maintaining themselves even in the absence of effective premating isolating mechanisms. Several alternative hypotheses for the evolution of the karyotypic forms and the establishment of the contact zone are discussed.

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NOTES:

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² One individual in the sample of 37 from the SW Llano Grande area is heterozygous at both *Ldh-2* and *Got-1*. (Its karyotype was not determined.) If alleles at these loci assort independently, as the data in [Table 4](#) seem to indicate, and the *Ldh-2^b* and *Got-1^d* alleles are actually segregating in the SW Llano Grande population at the observed low frequency (.014), the chance of both alleles occurring in the same genome is only .0002. This probability would be higher for recently introgressed alleles only a few generations removed from the original hybridization, but, even so, it is much more likely that the individual was an immigrant F₁ hybrid or backcross that had wandered into the collection area from the hybrid zone, possibly from the other side of Highway 190D via a culvert (Fig. [3B](#)). (A low population density in the SW Llano Grande area caused by our collecting in 1968 and 1969 may have encouraged immigration from surrounding areas.) For this reason, we have excluded this individual from consideration in our analysis.

³ For the sake of convenience, we here refer to chromosome 6 as a marker "locus." Similarly, chromosome 1 will be considered a "locus" polymorphic for two "alleles" in P1 populations, respectively the metacentric and fissioned conditions.

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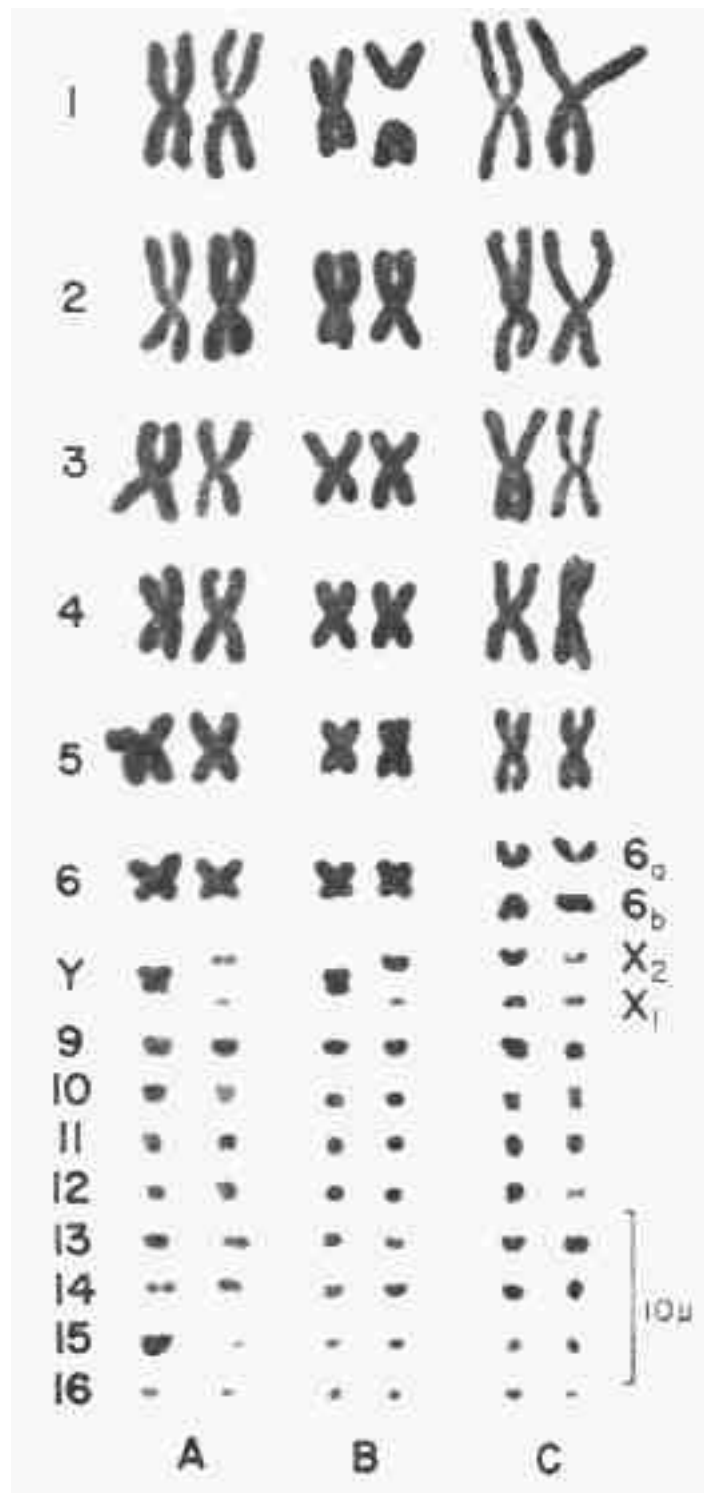
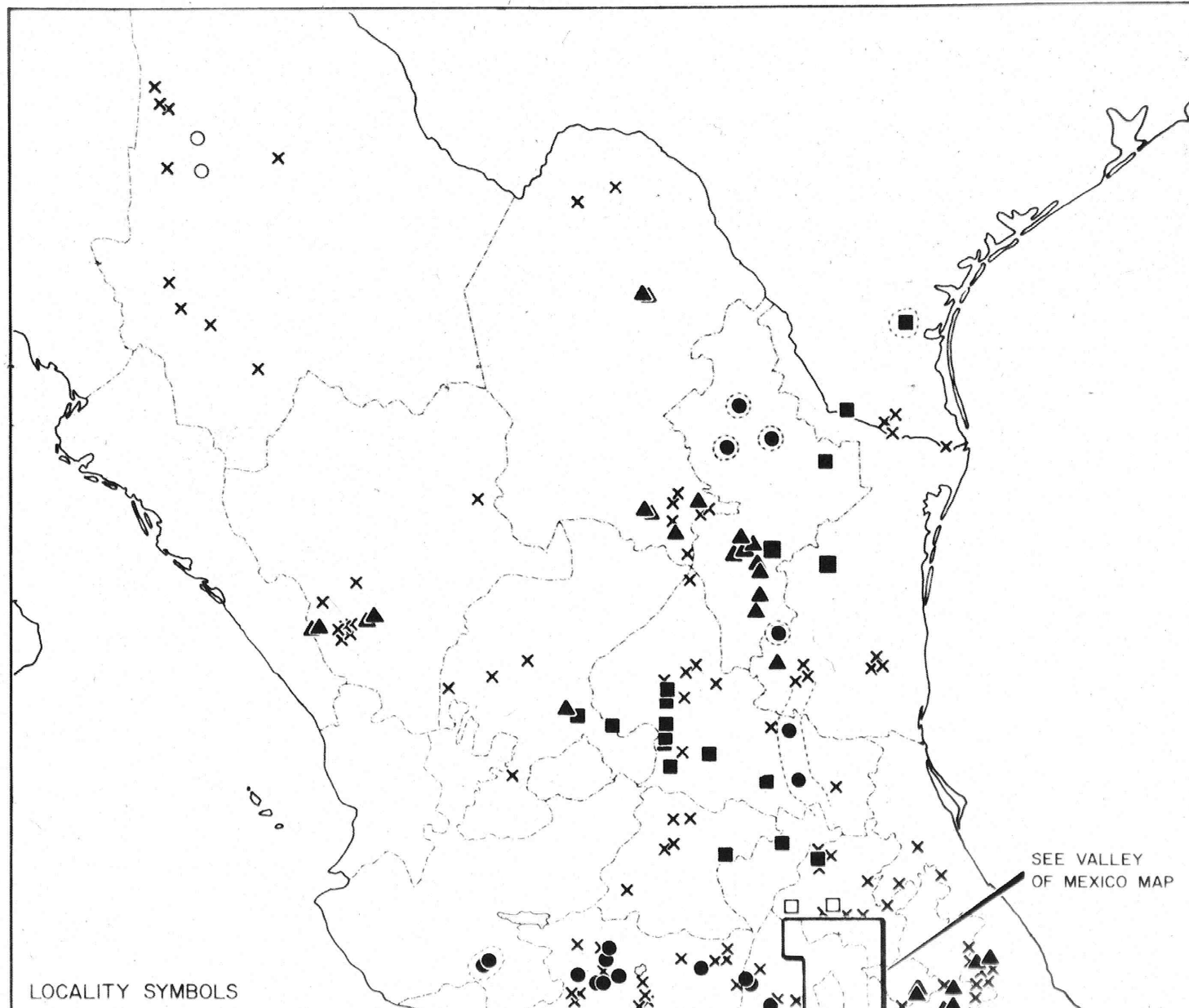


FIG. 1. Representative karyotypes of three *Sceloporus grammicus* populations. A. Standard; B. Polymorphic-1, heterozygous for a centric fission of chromosome 1--the Fis-1 mutation; C. Fission-6, homozygous for a centric fission of chromosome 6--the Fis-6 mutation.



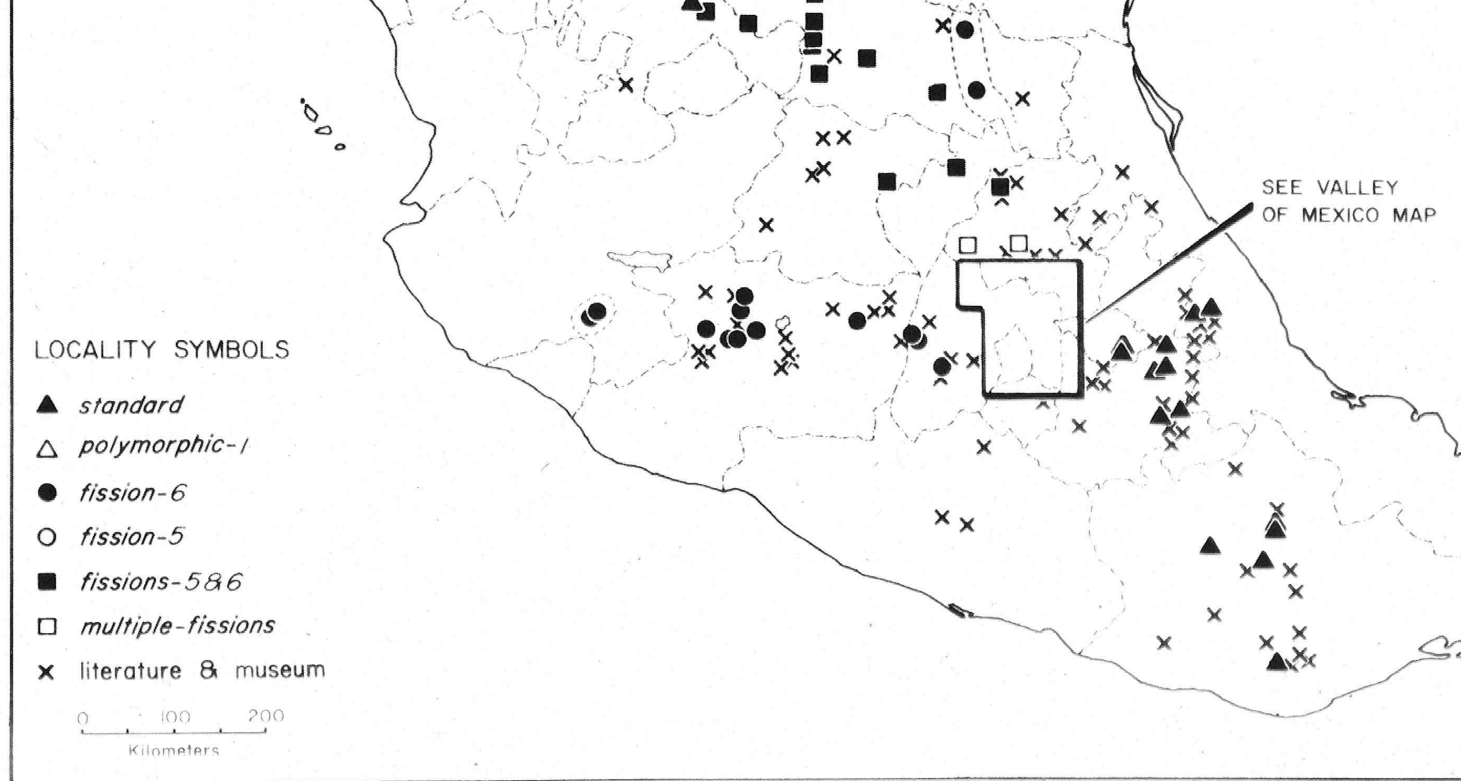
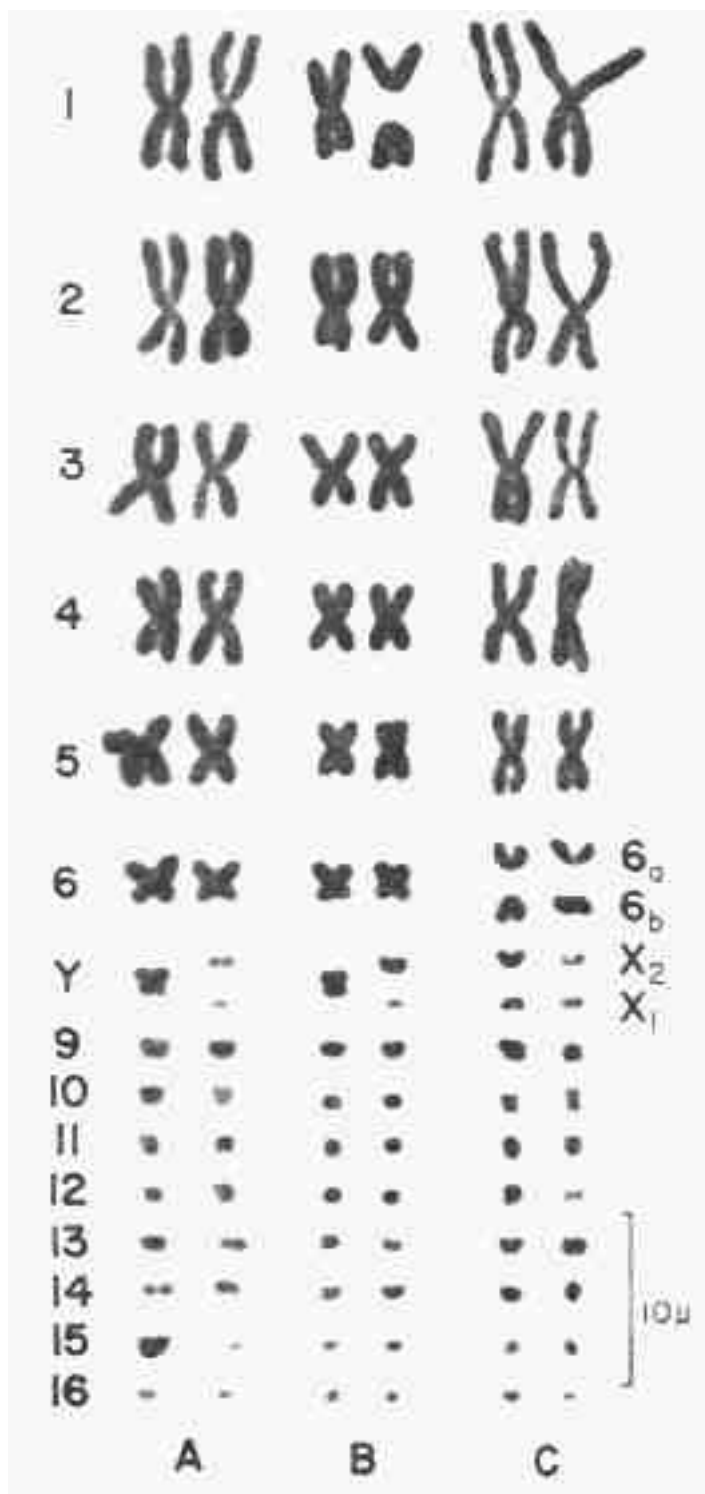
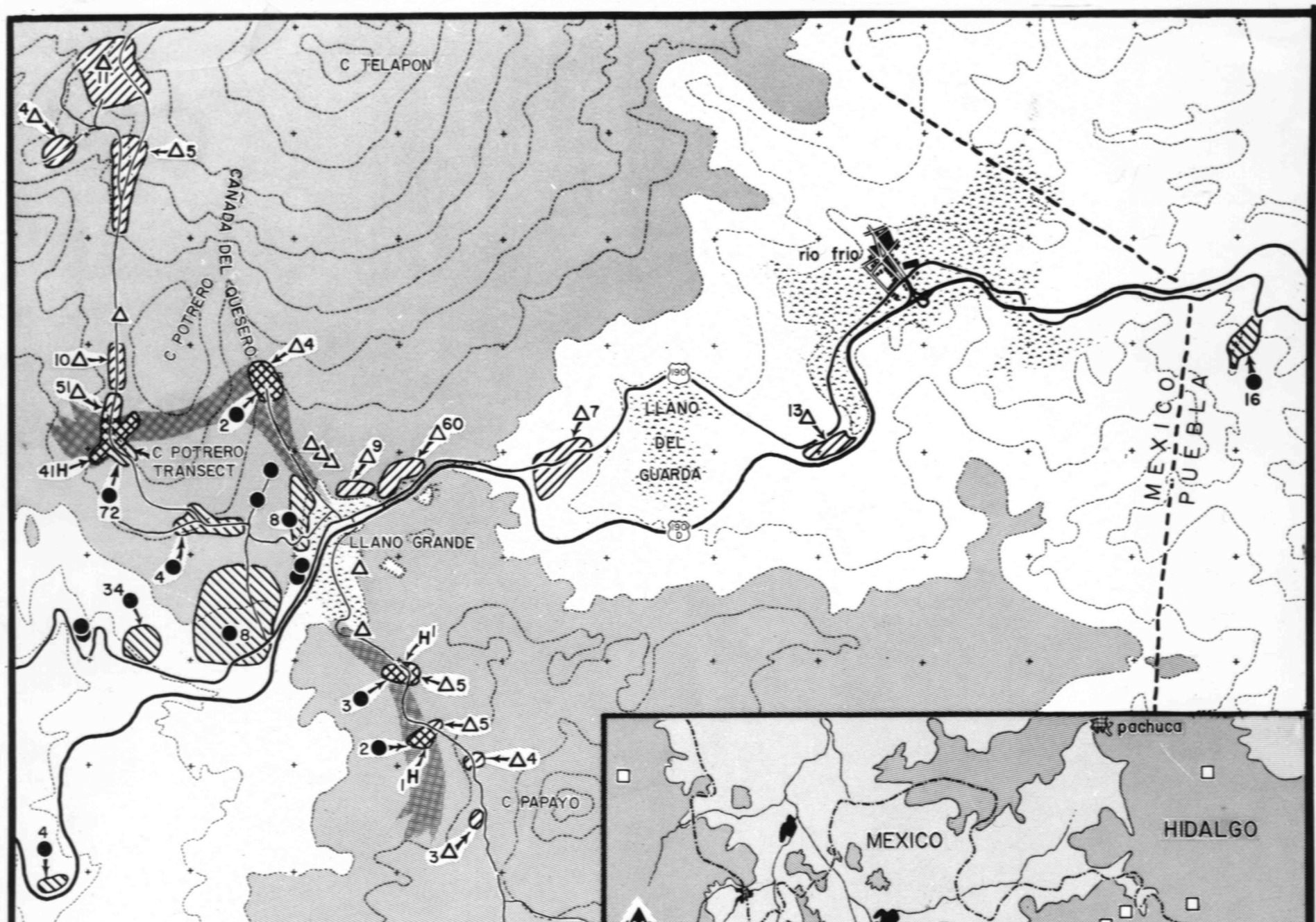


FIG. 2. Distribution of karyotypic forms of *Sceloporus grammicus* in Mexico. Symbols representing small populations believed to be disjunct from others of similar karyotype are circled with dashed lines. Literature and museum records are included to provide a better impression of overall distribution, but an absence of records may merely reflect an absence of collecting efforts in an area.





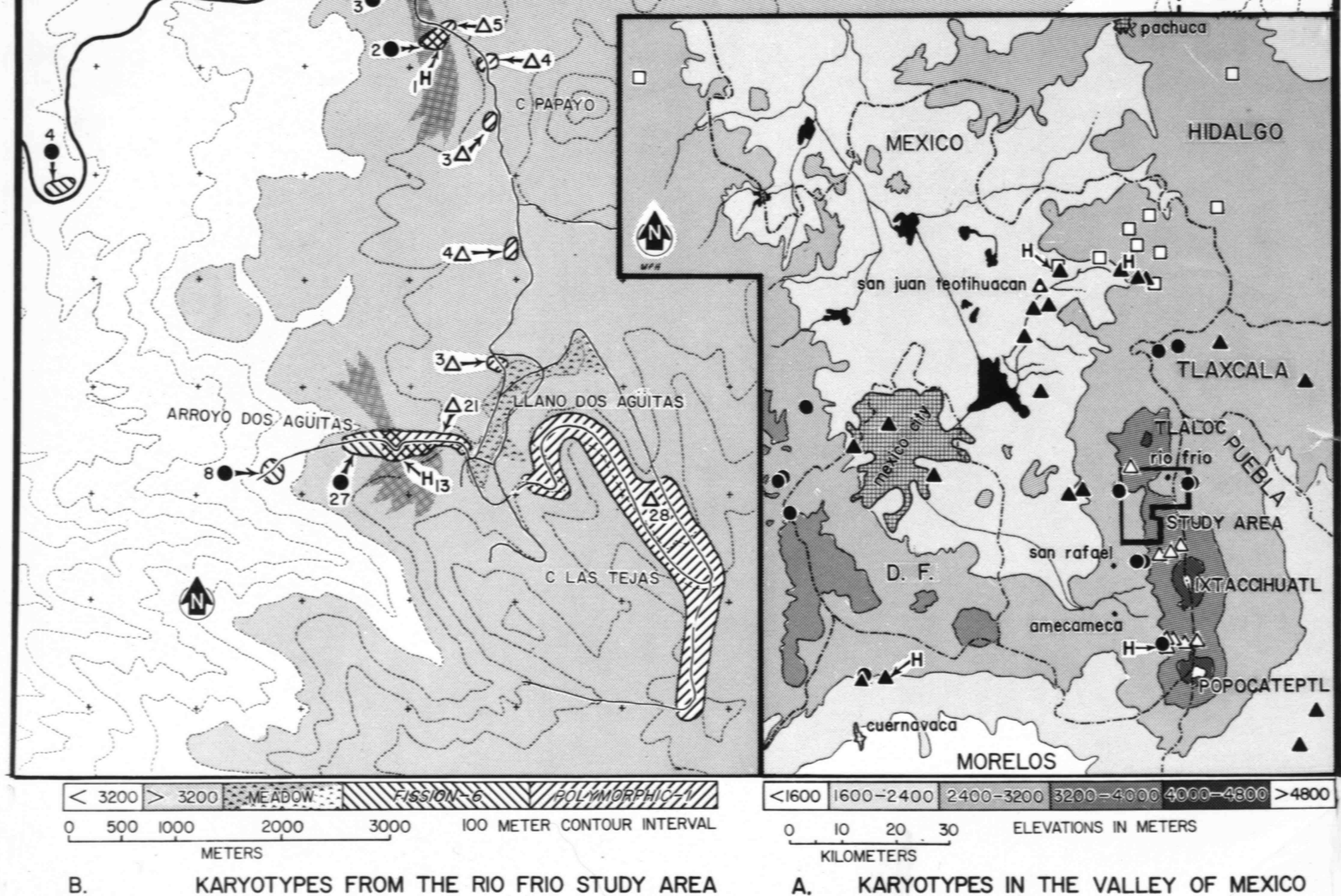


FIG. 3. Distribution of the karyotypic forms of *Sceloporus grammicus* in the Valley of Mexico region. A. The Valley of Mexico. B. The Rio Frio study area. Locality symbols are the same as in Fig. 2. Areas where individuals with

hybrid karyotypes have been found are indicated with H's in A and with cross-hatching in B. In B the inferred position of the zone of hybridization is indicated by heavy shading. Numbers by locality symbols indicate number of specimens karyotyped.

TABLE 1. Allele frequencies in four populations of *Sceloporus grammicus*.

Locus	Allele	Polymorphic-1	Fission-6		
		NE Llano Grande (N=31)	SW Llano Grande (N = 36)	Cerro Garnica (N = 21)	Nevado de Colima (N = 30)
<i>Es-1</i>	<i>a</i>	0.03	0.67	0.70	1.00
	<i>b</i>	0.97	0.33	0.30	
<i>Ldh-1</i>	<i>a</i>		0.07	1.00	1.00
	<i>b</i>	0.95	0.93		
	<i>c</i>	0.05			
<i>Ldh-2</i>	<i>a</i>		1.00	0.55	
	<i>b</i>	1.00		0.45	1.00
<i>6Pdg-1</i>	<i>a</i>			0.03	
	<i>b</i>		0.04	0.03	
	<i>c</i>	0.98	0.96	0.95	0.93
	<i>d</i>	0.02			
	<i>e</i>				0.07
<i>Got-1</i>	<i>a</i>	0.02			
	<i>c</i>	0.02			
	<i>d</i>	0.97		0.11	0.02
	<i>e</i>		1.00	0.89	0.98
α <i>Gpd-1A</i>	<i>c</i>	0.98	1.00	1.00	1.00
	<i>d</i>	0.02			
α <i>Gpd-1B</i>	<i>a</i>	0.02	-	-	-
	<i>b</i>	0.98	-	-	-
<i>Trf-1</i>	<i>b</i>	0.05	0.79	0.24	
	<i>c</i>			0.02	
	<i>d</i>	0.95	0.21	0.74	0.94

	<i>e</i>				0.03
<i>Pgi-1</i>	<i>b</i>	1.00	1.00	0.95	1.00
	<i>c</i>			0.02	
	<i>e</i>			0.02	
<i>Adh-1</i>	<i>b</i>				0.02
	<i>c</i>	1.00	1.00	1.00	0.98
<i>Pgm-1</i>	<i>b</i>	0.03	0.49	0.23	
	<i>c</i>	0.97	0.51	0.77	0.97
	<i>d</i>				0.03
<i>Ipo-1</i>	<i>a</i>	1.00	0.56	0.45	1.00
	<i>b</i>		0.44	0.55	
<i>Alb-1</i>	<i>a</i>		0.03	-	-
	<i>b</i>	0.98	0.50	-	-
	<i>c</i>		0.35	-	-
	<i>d</i>	0.02	0.11	-	-
	<i>e</i>		0.01	-	-

Eight monomorphic loci: *Mdh-1*, *Mdh-2*, *Idh-1*, *Got-2*, *Hb-1*, *Hb-2*, *Prot-A*, *Prot-B*.

TABLE 2. Genic variability in *Sceloporus grammicus* (19 loci).

Population	Number of individuals	Proportion of loci	
		Polymorphic per population ¹	Heterozygous per individual ²
<i>Polymorphic-1</i>			
1. NE Llano Grande	31	0.11	0.024
<i>Fission-6</i>			
2. SW Llano Grande	36	0.26	0.105
3. Cerro Garnica	21	0.42	0.134
4. Nevado de Colmia	30	0.05	0.017

¹ Loci are considered polymorphic if the frequency of the commonest allele ≥ 0.95 .

² Calculated from allele frequencies (Table 1).

TABLE 3. *Coefficients of genetic similarity between populations of Sceloporus grammicus (20 loci).*

	1 NE Llano Grande P1	2 SW Llano Grande F6	3 Cerro Garnica F6	4 Nevado de Colima F6
1	1.00	0.73	0.79	0.84
2		1.00	0.92	0.84
3			1.00	0.90
4				1.00

TABLE 4. *Variation in karyotypic characters and allele frequencies in Rio Frio populations of Sceloporus grammicus.*

	Population or genotypic class	N	Fixed differences			Polymorphic differences				
			<i>Fis-6</i>	<i>Ldh-2</i>	<i>Got-1</i>	<i>Fis-1^a</i>	<i>Pgm-1^b</i>	<i>Ipa-1^a</i>	<i>Es-1^b</i>	<i>Alb-1^b</i>
‘Pure’ P1	Cerro Las Tejas	28	-/-	b/b	d/d	—	0.00	0.98	0.98	0.98
	NE Llano Grande	31	-/-	b/b	d/d	—	0.03	1.00	0.97	0.98
	Cerro Potrero	34	-/-	b/b	d/d	0.15	0.06	1.00	0.94	0.98
Backcross to P1		6	-/-	a/b	d/d	0.17	0.00	0.92	0.92	1.00
		8	-/-	b/b	e/d	0.13	0.00	0.94	1.00	1.00
		6	+/-	b/b	d/d	0.17	0.00	0.75	0.92	0.83
	Sum: 1 locus het.	20				0.15	0.00	0.88	0.95	0.95
		1	-/-	a/b	e/d	0.00	0.00	1.00	1.00	1.00
		1	+/-	b/b	e/d	0.00	0.00	1.00	0.00	1.00
		7	+/-	a/b	d/d	0.07	0.14	0.64	0.56	0.71
Sum: 2 loci het.	9				0.06	0.11	0.72	0.63	0.79	
F ₁ Hybrids: 3 loci het.	13	+/-	a/d	e/d	0.00	0.31	0.65	0.60	0.77	
F ₆		1	+/-	a/b	e/e	0.00	0.50	0.50	0.00	0.50
		8	+/-	a/a	e/d	0.06	0.31	0.44	0.52	0.69
		5	+/+	a/b	e/d	0.00	0.40	0.70	0.00	0.80

	Sum: 2 loci het.	9				0.06	0.11	0.72	0.63	0.79
F ₁	Hybrids: 3 loci het.	13	+/-	<i>a/d</i>	<i>e/d</i>	0.00	0.31	0.65	0.60	0.77
Backcross to F ₆		1	+/-	<i>a/b</i>	<i>e/e</i>	0.00	0.50	0.50	0.00	0.50
		8	+/-	<i>a/a</i>	<i>e/d</i>	0.06	0.31	0.44	0.52	0.69
		5	+/+	<i>a/b</i>	<i>e/d</i>	0.00	0.40	0.70	0.00	0.80
		Sum: 2 loci het.	14			0.04	0.36	0.54	0.45	0.71
		1	+/-	<i>a/a</i>	<i>e/e</i>	0.00	1.00	0.50	1.00	1.00
		5	+/+	<i>a/a</i>	<i>e/d</i>	0.00	0.80	0.50	0.49	0.50
"Pure" F ₆		7	+/+	<i>a/b</i>	<i>e/e</i>	0.00	0.36	0.57	0.00	0.50
		Sum: 1 locus het.	13			0.00	0.58	0.54	0.46	0.54
		Cerro Potrero	50	+/+	<i>a/a</i>	<i>e/e</i>	0.00	0.59	0.37	0.21
	SW Llano Grande	36	+/+	<i>a/a</i>	<i>e/e</i>	—	0.49	0.56	0.33	0.50